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Cholera: An Overview of a Disease

Ezriel Leifer

Abstract

Although the disease, cholera, has been recognized since antiquity, the bacteria responsible for causing it was only discovered in the mid-19th century. Since 1817, cholera has spread on a global basis to cause seven pandemics. According to information reported to the World Health Organization in 1999, almost 8,500 people died and another 223,000 became sick with cholera worldwide. During the period between full outbreaks, the cholera organism, *Vibrio cholerae*, thrives in brackish waters, in harmless as well as disease-causing forms. *Vibrio cholerae* is just one of a variety of ocean-borne microbes that can sicken humans via seafood, drinking water, and swimming. Location, time, and intensity of cholera epidemics can now be accurately predicted from satellite observations of sea surface temperature, sea surface height, and chlorophyll in the water. Bacteria such as *Vibrio cholerae* have been found to be able to communicate with members of their own species and others to coordinate their behavior in response to cell density in a process known as quorum sensing, which relies on the production of and sensitivity to one or more secreted signal molecules. A growing body of scientific studies has identified a complex quorum sensing network in the human pathogen *Vibrio cholerae*. To gain a better understanding of this pathogen, this study provides an overview of the bacterium *Vibrio cholerae*, the mechanism of its virulence, a discussion concerning the symptomatology of the bacterium and its epidemiology. An analysis of how quorum sensing influences the virulence of the bacterium is followed by a discussion of diagnostic and treatment considerations. A discussion of ongoing preventative measures is followed by a summary of the research and salient findings in the conclusion.

Introduction

As the current swine flu epidemic reaches pandemic proportions, attention has been drawn away from yet another continuing threat to global health in the form of *Vibrio cholerae* and the cholera disease it can cause. Despite this inattention, the threat of cholera remains, and researchers are actively involved in investigating its etiology to identify the best practices in its prevention and treatment. To gain a snapshot of the historic and current efforts to combat this disease, this study provides an overview of the bacterium, *Vibrio cholerae*, the mechanism of its virulence, a discussion concerning the symptomatology of the bacterium and its epidemiology. An analysis of how quorum sensing influences the virulence of the bacterium is followed by a discussion of diagnostic and treatment considerations. A discussion of ongoing preventative measures is followed by a summary of the research and salient findings in the conclusion.

Review and Analysis

Overview of the Bacterium, Vibrio Cholerae

The cholera disease that is caused by *Vibrio cholerae* has been a source of dread for mankind since the first recorded pandemic in 1817 because of the high death rate associated with it and the rapidity with which it spreads. To date, about 200 O antigens have been distinguished serologically; however, just O1 and O139 have been found in epidemic and pandemic cholera isolates (Salim, Lan & Reeves, 2005). One of the preeminent researchers into the spread of cholera, Colwell, reports that, "Cholera, a diarrheal disease, has been with us for a very long time, even being mentioned in ancient Sanskrit writings. A medical textbook published in 1875 reported cholera to be a global pandemic, consistently appearing in

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India, Bangladesh, Latin America, and Africa. Today, cholera remains a serious problem” (2006, p. 753). The bacterium that causes cholera was first identified in 1854 and has continued to be investigated since that time (Faruque & Nair, 2008).

This scientific interest has been fueled in large part by the fact that the disease has become prevalent worldwide rather than being restricted to the Indian subcontinent as in years past. In this regard, Colwell (2006) reports that, “Until the nineteenth century, cholera was generally confined to the Indian subcontinent, but it then began to appear in Europe and the Americas as well. Since 1817, Western medical history describes seven global pandemics of cholera that have spread illness and death around the world. “The second of these seven pandemics reached the United States in 1832, traveling from New York to Philadelphia in a couple of weeks, and then cases appeared along the Atlantic coast all the way to the Gulf of Mexico” (Colwell, 2006). Likewise, according to Osborne (2008), “The pandemic that swept North American cities in 1832 was caused by a comma-shaped bacterium, *Vibrio cholerae*, known by various names including Asiatic cholera and Cholera morbus. It is now generally accepted that *Vibrio cholerae* originated in India, where cholera was endemic, and first reached the West in the early nineteenth century”.

In their account of historic efforts to combat the spread of cholera, Albertine, Persily and Riegilman (2007) repeat a well-known story thusly: “In 1855, pioneering epidemiologist John Snow traced the emergence of cholera in London to the public water supply. He persuaded authorities to remove the pump handle on the Broad Street well and thereby slowed and then stopped an outbreak of cholera”. It should be noted that Colwell (2006), though, suggests that the outbreak was stopped as a result of the operation of natural forces more than this

straightforward public health tactic, but the fact remains that the bacterium has been with mankind for millennia and efforts to stop its spread remain an ongoing source of investigation throughout the world.

The seventh pandemic (1961-present) is still widespread and has a severe impact on three different continents. The sixth pandemic ended in 1923, but the clone persisted at least until the 1990’s (Safa, Bhuiyan, Alam, Sack & Nair, 2005). In addition, a number of cholera outbreaks were reported after the sixth pandemic retreated, but before the start of the seventh pandemic. Isolates from these outbreaks were recognized as different from those of the sixth pandemic and were allocated to the El Tor biotype, while the sixth and fifth pandemics, both of which had been studied microbiologically, were referred to as the classical biotype (Safa et al.).

The outbreaks of the El Tor biotype took place in Indonesia and the Middle East (1926-1960) and are frequently described as being prepandemic isolates because they were later viewed as predecessors of the subsequent seventh pandemic, an episode that is also of the El Tor biotype (Safa et al.). Today, the environmental aspects of *V. cholerae* have been the focus of a considerable amount of research, and the major components of the El Tor phenotype have been shown to be present in most environmental isolates; the classical biotype is thought to have emerged through the loss of characters that are otherwise widespread in the species (Safa et al.). In addition, instances of sporadic indigenous cholera have been detected in Australia as well as the United States, both of which have been of the O1 El Tor biotype (Safa et al.). These are generally referred to as the US Gulf and Australian clones. All of the pathogenic forms discussed above had the O1 serotype, but in 1992 a variant of the seventh pandemic appeared with a new O antigen, O139; this variant is known as *V. cholerae* O139 Bengal (Safa et al.).

Mechanism of Virulence for Vibrio Cholerae.

Adherence to intestinal cells. The infectivity of *V. cholerae* is related to its ability to defeat the protective qualities that are typically present in the intestinal cells (Sparling, 1985). Microbial adherence to epithelial cell surfaces has been implicated as the first step in the initiation of several infectious diseases. Subinhibitory concentrations of antibiotics affect the adherence properties of microorganisms in various ways. They can inhibit the expression of fimbriae and the synthesis of other surface components, and they may also cause the release of constituents from the cell. The ability of antibiotics to affect the properties of microbial adherence to cell surfaces may be an important criterion in selecting an antibiotic for therapy. The relationships of *V. cholerae* have been studied in several ways, but the most useful insights have come from multilocus enzyme electrophoresis and more recently by multilocus sequence analyses (Salim et al.). According to Paz and Broza, "*Vibrio cholerae* bacteria are common hitchhikers attached to the surface of adult nonbiting midges (observed mainly with *Chironomus* sp., family Chironomidae). Both males and females have been reported to carry *Vibrio cholerae* strains that remain viable and culturable even after 14 days". In fact, researchers have been investigating the ability of *Vibrio cholerae* to adhere to animal cells and different ligands involved in intestinal colonization for some time, including virulence-associated toxin co-regulated pilus (TCP), outer membrane proteins, and lipopolysaccharides (Zampini et al. 2006). According to these researchers, "Its interactions with substrates present in the aquatic environment have been described more recently. *V. cholerae* O1 El Tor does not use TCP to form a biofilm on abiotic surfaces (borosilicate and cellulose), but rather the mannose-sensitive hemagglutinin (MSHA) pilus, which has no role in pathogenicity" (p. 267). The ability of *V. cholerae* to attach to chitin

has been demonstrated to be independent of the MSHA pilus, a finding that indicates divergent pathways for biofilm formation on nutritive and nonnutritive abiotic surfaces (Zampini et al.). Researchers have also determined that MSHA is involved in *V. cholerae* O1 El Tor and O139 adhesion to the exoskeleton of the planktonic crustacean *Daphnia pulex* and other ligands are believed to be used by *V. cholerae* O1 classical strains for zooplankton adhesion (Zampini et al.). In addition, *V. cholerae* O1 classical strain membrane proteins have been found to mediate N-acetyl glucosamine (GlcNAc)-sensitive attachment to chitin particles in vitro (Zampini et al.).

Release of toxins and their operation. The fatal effects of the disease are mainly due to the cholera toxin produced by specific strains of *Vibrio cholerae* (Paz & Broza). According to DNA researcher Lang, "Instead of having one circular chromosome, like most bacteria, the organism has two. The larger chromosome, comprising nearly three million base pairs, contains most of the organism's critical genes, including those coding for the disease-causing toxins and proteins that carry out essential cell functions" (2000, p. 39). The ability of *V. cholerae* to adhere to epithelial cell surfaces and release these disease-causing toxins has been cited as the first step in its infectivity (Shibl, 1985). In this regard, Todar (2009) reports that, "Cholera toxin activates the adenylate cyclase enzyme in cells of the intestinal mucosa leading to increased levels of intracellular cAMP, and the secretion of H_2O , Na^+ , K^+ , Cl^- , and HCO_3^- into the lumen of the small intestine. The effect is dependent on a specific receptor, monosialosyl ganglioside (GM1 ganglioside), present on the surface of intestinal mucosal cells" (p. 3). The specific manner in which *V. cholerae* operates is described by this clinician thusly: "The bacterium produces an invasin, neuraminidase, during the colonization stage which has the interesting property of degrading gangliosides to the monosialosyl form, which is the specific receptor for the toxin" (Todar, p. 3). The toxin released by *V. cholerae* contains (a) 5 binding (B) subunits of 11,500

daltons, (b) an active (A1) subunit of 23,500 daltons, and (c) a bridging piece (A2) of 5,500 daltons; the latter links A1 to the 5 binding subunits (Todar). After *V. cholerae* has penetrated the cell, the active subunit transfers ADP ribose from NAD^+ to a protein (called Gs or Ns) enzymatically; this process controls the adenylate cyclase system situated on the interior of the plasma membrane of mammalian cells (Todar). According to Todar, "Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl

moiety of NAD^+ to a component of the adenylate cyclase system. The process is complex. Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP; however activation is normally brief because another regulatory protein (Gi), hydrolyzes GTP”.

Typically, the active subunit fragment changes the attachment of ADP-Ribose (ADPR) to the regulatory protein thereby creating Gs-ADPR, a protein from which GTP is unable to be hydrolyzed. Because GTP hydrolysis serves to shut down the adenylate cyclase process, the enzyme continues to be active in the cell (Todar). As a result, the bottom-line impact of the toxins released by *V. cholerae* are to cause cAMP to be produced at an inordinately high rate, a process that in turn stimulates mucosal cells to discharge large amounts of Cl^- into the contents of the intestine (Todar). Thereafter, H_2O , Na^+ and other electrolytes are produced as a result of the osmotic and electrical gradients that are the result of the loss of Cl^- (Todar). In sum, then, “The lost H_2O and electrolytes in mucosal cells are replaced from the blood. Thus, the toxin-damaged cells become pumps for water and electrolytes causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera” (Todar, p. 3). Neither *Vibrio cholerae*, though, nor the toxins it releases are capable of passing through the gut wall (Gibbs, 2005).

Symptomology of Vibrio Cholerae.

Accounts of the symptoms associated with the 1832 North American pandemic were grisly in their descriptions. For instance, Osborne reports that “Victims of the disease were stricken with severe diarrhea and vomiting, accompanied by excruciatingly painful cramps through the trunk and legs. As dehydration continued, the bodily fluids were excreted as ‘rice water’ and the victim quickly collapsed and turned blue. “Often, fifty per cent or more of those who caught cholera died, with death coming to the more fortunate victims in as little as four to six hours “(p. 30). Empirical accounts from the 1832 North American pandemic described one victim as being: “. . . a young woman of apparently twenty-five ... absolutely convulsed with agony. Her eyes were started from their sockets, her mouth foamed, and her face was a frightful livid purple. She had been taken in perfect health only three hours before, but her features looked to me marked with a year of pain. The first attempt to lift her produced violent vomiting, and I thought she must die instantly” (A walk through a cholera hospital, 1832, p. 37).

Epidemiology of Vibrio Cholerae.

One of the first aspects that researchers learned about cholera was about how the disease was transmitted: “The disease was transmitted through human feces, generally ingested in drinking water. It first escaped the Indian subcontinent in 1817, reaching Moscow in September 1830 and thence westward across Europe. “The mysterious origins and terrifying nature of the disease added to the sense of dread it created in Europe and North America” (Osborne, p. 30). In 2000, scientists identified the entire order of paired chemical building blocks that constitute the DNA of the deadly cholera bacterium, *Vibrio cholerae*, and found that it is “a comma-shaped microbe [that] causes a severe diarrheal disease that has been endemic in southern Asia for at least 1,000 years” (Lang, 2000). Cholera continues to represent an important cause of morbidity and

mortality in many regions of the world, and there is currently a high incidence of fresh outbreaks in Africa (Paz & Broza, 2007).

The transmission of cholera epidemics may also be related to the dominant wind direction over land (Paz & Broca, 2007). These researchers examined the geographic diffusion of three cholera outbreaks through their linkage with the wind direction: a) the progress of *Vibrio cholerae* O1 biotype El Tor in Africa during 1970-1971 and b) again in 2005-2006; and c) the rapid spread of *Vibrio cholerae*

O139 over India during 1992-1993. In addition, it is possible that the influence of the wind direction on windborne dissemination by flying insects, which may serve as vectors, plays a role. The analysis of air pressure data at sea level and at several altitudes over Africa, India, and Bangladesh by Paz and Broca found a correspondence between the dominant wind direction and the intracontinental spread of cholera. According to Holzman (2007), *Vibrio cholerae* is just one of a variety of ocean-borne microbes which can sicken humans via seafood, drinking water, and swimming. Location, time, and intensity of cholera epidemics can now be accurately predicted from satellite observations of sea surface temperature, sea surface height, and chlorophyll in the water (Foster, 2000). Likewise, Colwell (2006) notes that, "In 1977, my coworkers and I reported that *Vibrio cholerae*, the causative agent of cholera, could be cultured from Chesapeake Bay water samples. It was the first report of the isolation of the *V. cholerae* from noncholera-endemic geographical areas; cholera had not been reported in Maryland since the 1900s. "It was difficult for us to make our case, namely that the cholera vibrio was a native inhabitant of the Chesapeake Bay, since cholera had not occurred in the region.

Despite this lack of evidence, though, these researchers did in fact identify *Vibrio cholerae* in the Chesapeake Bay and thereafter applied molecular techniques to demonstrate that

the bacterium is naturally occurring in the aquatic environment, with annual peaks in the spring and fall. In addition, Colwell and her colleagues found that the *V. cholerae* is associated with plankton. We now know that river, estuary, and coastal waters are reservoirs of these bacteria globally, but their data showing an environmental source of the cholera bacteria implied a paradigm shift for the medical community. "It has taken about 20 years for the paradigm change of cholera being transmitted only by person-to-person contact to the recognition that the cholera vibrio exists in the environment as a natural inhabitant (p. 753). In addition, Colwell and her associates determined that the bacterium experiences a dormant phase between outbreaks and epidemics and through the use of gene probe molecular techniques were able to prove its year-round presence in the environment.

As noted above, *V. cholerae* O1 classical strains adhere to zooplankton, and Colwell and her colleagues determined that this relationship with zooplankton was particularly important. According to these researchers, "In the spring, when the water warms, phytoplankton become abundant; using sunlight for energy, the population of phytoplankton increases significantly. That population increase is followed by blooms of zooplankton, the miniature 'cattle' of the sea, which graze on the phytoplankton. We were able to show a relationship of sea surface temperature increase with onset of cholera epidemics because of the fact that vibrios comprise the natural microbial flora of zooplankton, the populations of which increase spring and fall in annual cycles. The seasonal pattern of cholera follows the seasonal rise and fall in sea surface temperature and height (Colwell).

In 1991-1992, a massive cholera epidemic took place in Peru and about 200,000 cases and 5,000 deaths were reported as a result of this epidemic; this was an unprecedented occurrence because cholera had not been reported in South America for almost a century

(Colwell). Moreover, the epidemic took place during a period of a strong El Nino climatic event. Based on climatologists' prediction concerning another comparable El Nino event in 1997-1998, Colwell and her associates hypothesized that there was a linkage of the 1991-1992 cholera epidemic with El Nino and predicted additional cholera outbreaks would occur in 1997-1998. As Colwell notes, "With colleagues from Peru, Chile, Ecuador, Brazil, and Mexico, we conducted a training session on molecular techniques for direct detection of the *V. cholerae* in water and plankton. As the sea surface temperatures in these Latin American countries increased in 1997 because of El Nino, the team was able to detect the presence of the cholera bacteria associated with plankton, with numbers of the bacteria increasing from spring to summer (September 1997 to March 1998) and cases of cholera occurring in late November through the summer of 1998" (p. 753).

Colwell and her colleagues were able to conclude that El Niño is another important climate factor related to cholera, notably in cholera-endemic countries. The cases of cholera in Peru in 1997-1998 were directly correlated with sea surface temperature. The relationship of the disease with this climate factor was statistically significant. In Bangladesh and other countries where severe cholera epidemics occur, such as Peru, Indonesia, and India, the influence of monsoons or severe weather is important. Matlab, near Dhaka, Bangladesh, comprises a "hotspot" of cholera. The villages are constructed around bodies of water, and specific locations of epidemics have been determined. Research on cholera has been conducted in Matlab by Colwell et al. since 1975. The influence of the Himalayas on the weather in Bangladesh is significant, because the monsoon rains wash nutrients into the rivers and ponds. Typically, houses in Bangladesh are located at the edge of a pond, from which villagers draw their water for household use. A definable relationship between sea surface temperatures, sea surface height, and cholera epidemics was established and published in the proceedings of the National Academy of Science (Lobitz et al., 2000). Taken together, the complex factors of sea surface temperature, sea surface height, and zooplankton populations provide a predictive capacity for cholera epidemics in developing countries that derives from climate monitoring through satellite sensors (Colwell, 2006).

Finally, with the assistance of sociology researchers working in Bangladesh, they were able to test the hypothesis they constructed: that if they could remove zooplankton from the water the villagers used to meet household needs, the incidence of cholera could be reduced. With a very simple filtration technique that Colwell et al. devised using sari cloth folded in 4 layers, the researchers were able to reduce cholera by approximately 50 percent in villages where families had been instructed in the filtration method. The complex of plankton, people, and climate, together with a simple solution based on science (under the electron microscope, the folded sari cloth could be seen to provide a 20 micron filter--and the zooplankton range in size roughly 50 to 200 microns), provides the interrelationship that allows an understanding of a global infectious disease--an understanding that would not otherwise be possible.

Moreover, studies conducted by the Smithsonian Environmental Research Center in recent years have also determined that cholera is far more prevalent in ballast water than previously thought; a sampling of ballast water from fifteen vessels entering Chesapeake Bay identified one strain of cholera, *Vibrio cholerae* O1, on every ship surveyed. In addition, a different strain of cholera, *Vibrio cholerae* O139, which had been previously unidentified in the United States, was identified in the ballast water of fourteen of the fifteen vessels surveyed (Foster, 2000).

How Quorum Sensing Influences the Virulence of the Bacteria Vibrio Cholerae.

According to Cámara, Hardman, Williams and Milton (2002), "Bacteria can communicate with members of their own species and others to coordinate their behavior in response to cell density. This phenomenon, known as quorum sensing, relies on the production and sensing of one or more secreted signal molecules. A recent study identifies a complex quorum sensing network in the human pathogen *Vibrio cholerae* (p. 217). In this regard, Tsou, Cai, Liu, Zhu and Kulkarni (2009) report that, "The quorum-sensing pathway in *Vibrio cholerae* controls the expression of the master regulator HapR, which in turn regulates several important processes such as virulence factor production and biofilm formation. While HapR is known to control several important phenotypes, there are only a few target genes known to be transcriptionally regulated by HapR (p. 2747). In this study, Tsou and her colleagues combined bioinformatic analysis with experimental validation to discover a set of novel direct targets of HapR. The findings of the Tsou et al. study provide evidence for two distinct binding motifs for HapR-regulated genes in *V. cholerae* as follows. The first binding motif is similar to the motifs recently discovered for orthologs of HapR in *V. harveyi* and *V. vulnificus*; these results also demonstrate that this binding motif can vary in length in *V. cholerae*; the second binding motif shares common elements with the first motif,

but in contrast to the first binding motif, the second is fixed in length and does not have dyad symmetry at the terminal points (Tsou et al.).

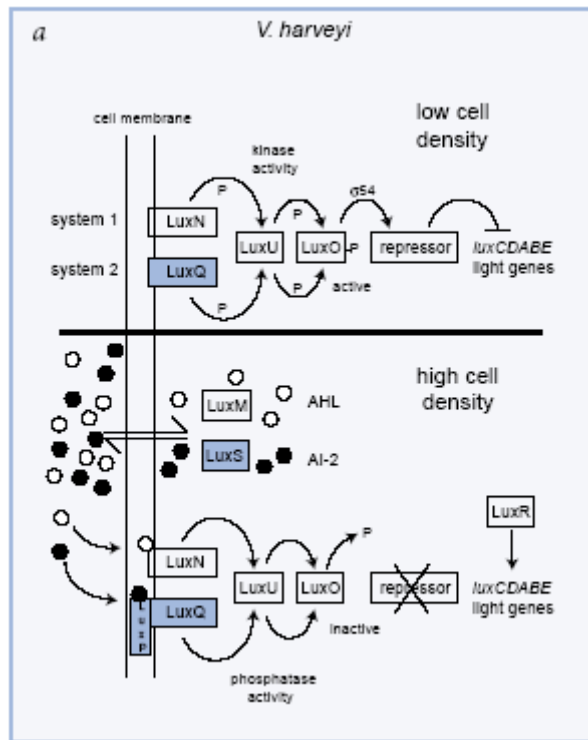


Figure 1. Quorum sensing in *Vibrio harveyi*.

Source: Camara et al.

In *V. harveyi*, a harmless marine bacterium closely related to *V. cholerae*, quorum sensing serves to activate bioluminescence (*luxCDABE*) at high cell density levels; to accomplish this, two parallel signaling systems are used to control light production. Likewise, as shown in Figure 2 below, quorum sensing in *V. cholerae* functions by restricting virulence gene expression at high cell density through the use of three parallel signaling systems as follows:

1. System 1 includes CqsA, a putative synthase for the unidentified CAI-1 signal molecule, and CqsS, a hybrid sensor/kinase that responds to the CAI-1 signal.
2. System 2, LuxSPQ, is identical to that of *V. harveyi* (see above).
3. System 3 has not been characterized; however, this system is believed to respond to an intracellular signal and is therefore thought to be unrepresentative of a true quorum sensing system. System 3 does not seem to transmit its signal through LuxU but rather directly through LuxO, the pivotal regulator for all three systems. Like the *V. harveyi* system, LuxO is activated via phosphorylation at low cell density and, together with σ54, activates the expression of

an unidentified repressor that blocks the production of HapR, which acts as a repressor of virulence gene expression. As a result, at low cell density, virulence genes are expressed; at high cell density, though, LuxO remains inactive and cannot repress HapR. As HapR is now expressed, virulence gene expression is repressed (Camara et al.).

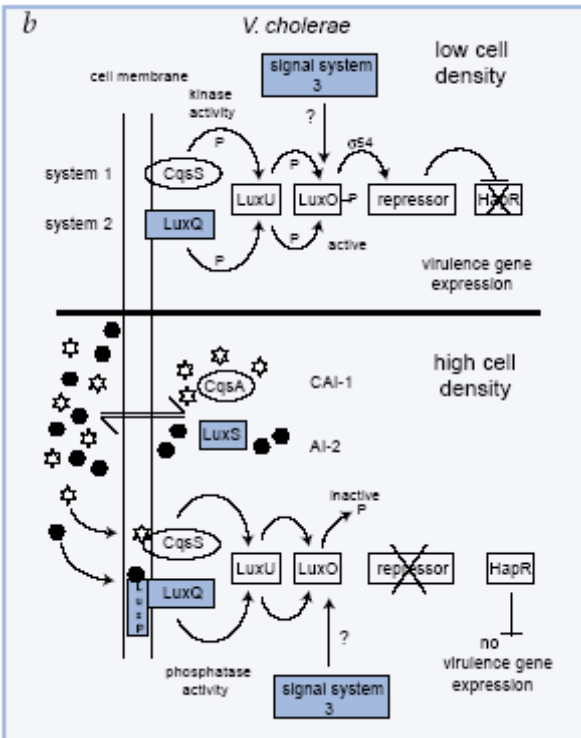


Figure 2. Quorum sensing in *Vibrio cholerae*.

Source: Camara et al.

The quorum sensing responses in *V. harveyi* and *V. cholerae* represent instances of complex cell-to-cell communication techniques that focus multiple cell-density-dependent, and perhaps independent, signals via an alternate regulatory pathway to regulate bacterial behavior (Camara et al.). According to these researchers, “Variations on the complexity or organization of these multiple-channel signaling systems may be forthcoming, as components of the *V. harveyi*-like signaling systems have been found in *V. fischeri* as well as the fish pathogen *Vibrio anguillarum*11–13; however, adding yet more complexity to the process, both of these organisms also possess LuxI/LuxR-type quorum sensing signaling systems” (Camara et al., p. 218). The coordination of a multi-cellular response to rapidly adapt to changes that take place in the surrounding environment is a useful survival strategy for pathogenic and non-pathogenic bacteria alike (Camara et al.). Just as other forms of life respond to the density levels of their populations in different ways, so too does the *V. cholerae*. In this regard, Camara and his associates report that, “Cell density is just one of a multitude of signaling parameters that a bacterium faces in any given environment, and coordination of all signals into a physiological response will require intersystem cross-talk. Incorporation of quorum sensing into a global network of regulatory responses may be required to increase selectivity and efficiency of a bacterium’s response to the stresses it encounters in its growth environment” (Camara et al., p. 218).

One unexpected outcome of a study by Miller et al. (2002) was that, in contrast to many other bacterial pathogens, quorum sensing in *V. cholerae* serves to repress, rather than activate, virulence gene

expression at high cell density levels in vitro (Camara et al.). A speculative model was advanced that indicated that following initial colonization (low cell density), LuxO represses HapR, which is also a repressor of virulence gene expression, thereby permitting colonization of the intestines and cholera toxin production. In this regard, Camara et al. add that, “As the bacterium multiplies, the quorum sensing signal molecules accumulate and activate the two quorum sensing systems, which inactivate LuxO. HapR is then produced, repressing virulence gene expression while activating the production of the protease, HapA”. (Camara et al., p. 218).

HapA is believed by scientists to promote the detachment of the bacteria from intestinal tissue, thus facilitating the spread of the pathogen. As there is still a third unidentified signaling system influencing gene regulation through the LuxO regulatory cascade, and additional regulators of virulence gene expression to be considered, the actual role of signaling systems 1 and 2 in the virulence of *V. cholerae* in vivo remains unclear. According to these researchers, “Nonetheless, the model proposed for *V. cholerae* should provide an exciting twist on the role of quorum sensing in virulence” (Camara et al., p. 218).

Finally, a study by Zhu, Miller, Vance, Dziejman, Bassler and Mekalanos (2002) notes that the production of virulence factors including cholera toxin and the toxin-coregulated pilus in the human pathogen *Vibrio cholerae* is strongly influenced by environmental conditions. These researchers report that, “The well-characterized ToxR signal transduction cascade is responsible for sensing and integrating the environmental information and controlling the virulence regulon” (p. 3129). In their timely study, “Quorum-sensing regulators control virulence gene expression in *Vibrio cholerae*,” Zhu et al. demonstrate that besides the known components of the ToxR signaling circuit, quorum-sensing regulators are involved in regulation of *V. cholerae* virulence. These researchers concentrated on the regulators LuxO and HapR because homologues of these two proteins control quorum sensing in the closely related luminous marine bacterium *Vibrio harveyi* in this study. According to Zhu and his associates, “Using an infant mouse model, we found that a LuxO mutant is severely defective in colonization of the small intestine. Gene arrays were used to profile transcription in the *V. cholerae* wild type and the LuxO mutant. These studies revealed that the ToxR regulon is repressed in the LuxO mutant, and that this effect is mediated by another negative regulator, HapR. We show that LuxO represses HapR expression early in log-phase growth, and constitutive expression of HapR blocks ToxR-regulon expression.

Additionally, LuxO and HapR regulate a variety of other cellular processes including motility, protease production, and biofilm formation. Together these data suggest a role for quorum sensing in modulating expression of blocks of virulence genes in a reciprocal fashion in vivo” (p. 3130).

Diagnostic and Treatment Considerations.

In many ways, *Vibrio cholerae* is an elusive bacterium that can elude ready detection that confounds diagnosis. In this regard, Henrickson, Wong, Allen, Ford and Epstein (2001) report that some gram-negative organisms such as *V. cholerae* are capable of adapting to low-nutrient environments through reductive division; in other words, although there is no change in total biomass, more organisms develop but they develop at a significantly reduced metabolic rate. When there are unfavorable environmental conditions, such as cold or reduced nutrients, *Vibrio cholerae* bacteria can shrink to 1/300th of its original size and enter a dormant state; remaining in the dormant state for extended periods of time, increasing in size when environmental conditions once again become favorable. Moreover, *V. cholerae* can become nonculturable on routine culture plates, but remain viable, and could regrow under appropriate conditions (Henrickson et al.).

As noted above, cholera has a rapid onset and most frequently occurs in epidemics that are spread through contaminated water. According to Long, “Oral rehydration is an effective treatment, but left untreated, cholera causes severe diarrhea that has a high mortality rate, particularly in young children” (p. 39). Even some simple precautionary methods can reduce the incidence of infection by *Vibrio cholerae*,

though. For instance, Holzman (2007) emphasizes that if people in the least stable situations—for example, in war-torn developing countries—are provided with sufficient warning, they could filter drinking water using low-tech devices, cutting

the infection rate by 50%. Researchers have successfully monitored and predicted cholera epidemics in Bangladesh through the use of satellites, as reported in the 15 February 2000 issue of *Proceedings of the National Academy of Sciences* (Holzman). In this regard, Dremeaux (2003) reports that, “Ingesting a high dose of the waterborne bacteria *Vibrio cholerae* O1 produces cholera, an infection that causes severe dehydration brought on by acute diarrhea and vomiting. Left untreated, cholera can kill a person in 24 hours. Filtering water with a folded piece of old cloth before drinking it cuts the rate of cholera contraction by half, according to a three-year study in 65 Bangladeshi villages published in January 2003 in the U.S. journal *Proceedings of the National Academy of Sciences*” (p. 9). According to Gibbs (2005), “Up to 90 per cent of cases of cholera will have only mild diarrhea. The remainder may exhibit severe watery diarrhea leading to dehydration. Since neither the bacteria nor its toxins can pass through the gut wall and into the blood, cholera does not increase the sufferer's temperature. In severe cases, a simple antibiotic such as Tetracycline will decrease the diarrhea. Rehydration is imperative. “Oral rehydration salts are usually sufficient, but severe cases will need intravenous fluids” (p. 101).

Discussion.

As noted above, simply filtering contaminated water with a folded piece of old cloth before drinking it reduces the rate of cholera contraction by fully 50 percent. According to Dremeaux (2003), “The finding has the potential to save thousands of lives annually. Fabric from saris, the flowing, colorful garments South Asian women often wear, was cheap and readily available to the 133,000 people who participated in the study, and comparable fabrics could function as filters for populations at risk for cholera around the world” (p. 9). The sari cloth does not trap *V. cholerae* per se, but rather the copepods, a type of zooplankton onto whose mouths, surfaces, and egg cases the vibrios attach (Dremeaux). While a majority of the vibrios did remain

free in the filtered water, their numbers were frequently diminished sufficiently to fall short of an infective dose, estimated at $[10^4]$ to $[10^6]$ *V. cholerae*; in fact, the dilution lowered the rate of cholera infection by almost half (48%). According to Dremeaux, “For those who did contract cholera via filtered water, the severity of the disease appears to have lessened, the report says. Electron microscopy had revealed that sari cloth, when folded four to eight times, would create a filter of approximately 20 mm pore size, removing all copepods--and the cholera-causing bacteria attached to them--from the water. The old saris used in the experiment were expected to be more effective than new ones, their laundered fabric resulting in a smaller pore size” (p. 9). Although clean potable water would be preferable of course, and boiled water would be an acceptable alternative, there remains a paucity of fuel for many villagers to use for this purpose. In this regard, Dremeaux reports, “Rivers and ponds are a common source of drinking water for the villages in rural Matlab, Bangladesh. Boiling water, which kills all waterborne microorganisms, is often impossible for the villagers, who are hard-pressed to find dry wood for fuel or the money to buy it. High concentrations of arsenic in the groundwater make well sources a poor alternative (Dremeaux, p. 9). Although nylon mesh filter was nearly as effective as the sari cloth in reducing cholera, that material is more costly and harder to find in rural Bangladesh than material from saris (Dremeaux). In sum, Colwell (2006) emphasizes that, “The interaction of humans, cholera bacteria, the zooplankton host of the bacterium (the copepod), and the environment in the case of cholera can be employed to make reasonable predictions about this climate-driven disease. The issues are truly international and represent those that comprise a global scientific enterprise and encompass many other infectious diseases” (p. 754).

Conclusion

The research showed that the disease caused by *Vibrio cholerae* has plagued mankind for thousands of years, but the pathogen that was responsible for causing cholera was only discovered a century and a half ago. Over the past two centuries, cholera has caused seven pandemics and has been responsible for tens of thousands of deaths. The research also showed that even during periods when it is dormant, *Vibrio cholerae*, continues to thrive in brackish waters in both harmless and disease-causing forms. Researchers have discovered that it is possible to predict with some accuracy future outbreaks of cholera epidemics using satellite observations of sea surface temperature, sea surface height, and chlorophyll in the water. Finally, the research also showed that bacteria such as *Vibrio cholerae* are capable of communicating with other members of their species in order to coordinate their behavior in response to cell density in a process known as quorum sensing that relies on the production and sensing of one or more secreted signal molecules. A growing body of scientific studies has identified a complex quorum sensing network in the *Vibrio cholerae* that may help researchers identify superior diagnostic and treatment protocols in the future, but in the meantime, there have been some commonsense approaches to treating water contaminated by *Vibrio cholerae* that reduce its incidence by almost half.

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